

EXHIBIT I

factor 3 in rAAV8 productivity was observed in the cell culture and was maintained after purification, compared to the recently developed production system based on the use of 2 baculoviruses (R. Kotin's lab). The produced rAAV8 capsids displayed a reduced degradation profile of the capsid proteins VP1/VP2 due to the elimination of the baculovirus cathepsin protease gene. This optimized system allows the production of an improved quantity of rAAV vectors with improved vector quality of high importance for pre-clinical and, in particular, for clinical use and represents a considerable advance in the improvement of this AAV manufacturing system.

Sensory (Ophthalmic and Auditory) Gene & Cell Therapy

53. Retinal Gene Therapy May Alter Connectivity of Visual Pathways

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**Introduction:** Leber's congenital amaurosis (LCA) is a blinding eye disease with no cure. Due to the relatively slow progression, LCA type 2 (LCA2), caused by mutations in the gene encoding retinal pigment epithelium 65 kDa protein (RPE65), has been considered for gene therapy (GT). At the Children's Hospital of Philadelphia, 12 LCA2 patients received unilateral GT resulting in dramatic enhancement of vision. Previously we reported the effect of GT on the function of the visual cortex using functional magnetic resonance imaging (fMRI). In the present study, we utilized diffusion tensor imaging (DTI) to assess the effect of GT on the brain's visual fiber pathways and examined the relationship between visual structure and function. **Methods:** Ten unilaterally treated LCA2 patients (8/10 Rt. eye treated) and ten controls underwent fMRI (>1.5 yrs post-surgery) and DTI (30 directions) in a 3T MR and a 32-channel head coil. A DTI population-based atlas was created to perform tractography. Major fiber bundles crossing the occipital cortex, such as the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, occipito-callosal fibers, optic radiation tracts (ORT) and optic chiasm were extracted. Integrity of fiber tracts was assessed by comparing the average of the fractional anisotropy (FAavg) of each tract between the LCA2 and controls. Group average fMRI (fMRIavg) for the right eyes (treated eyes) was obtained to compare group responses and to correlate the structure and function in LCA2. **Results:** The optic radiation tracts (ORT) were the only bundles showing differences among subjects with right FAavg similar among groups ( $p<0.5$ ) but the left displaying lower FAavg ( $p<0.005$ ) for LCA2 patients. The right eye fMRIavg response to checkerboard stimuli depicted symmetrically distributed activation for controls but significantly greater right distributed cortical activations for the LCA2 patients. Pearson correlations showed a strong coefficient ( $r=0.56$ ) between the FAavg of the right ORT and fMRIavg of the right visual cortex and ( $p=0.12$ ) for LCA2. The low significance level may be due to a small number of LCA2 participants treated in the same eye ( $N=8$ ). **Conclusions:** Given that LCA is a bilateral disease it is assumed to affect the brain's optic pathways symmetrically. Our results showed decrease in the FAavg for the left but not the right ORT. Consistent with tract results, the right eye fMRIavg also confirmed significant increased activation in the right visual cortex for LCA2. These results are coherent with the fact that LCA2 ( $N=8$ ) patients received sub-retinal injection in the superior temporal section of their right retina, primarily affecting the right visual pathways (e.g. Rt. ORT). The possible increased myelination (increased FA) of the right ORT may stem from experience-dependent structural plasticity as a result

of increased neuronal stimulation promoted by retinal gene therapy. This study, for the first time, reveals the powerful long-term effect of gene therapy on the structure of human visual fibers.

54. Gene Therapy for Wet-AMD: Progress Report on a Phase I/II Clinical Trial

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We developed a rAAV construct for the delivery and production of a potent naturally occurring anti-vascular endothelial growth factor (VEGF) protein, the soluble Fms-related tyrosine kinase-1 (sFlt-1) to re-establish the balance between VEGF and sFlt-1 in the retina. This paper aims to assess the safety, pharmacology and immunology of rAAV.sFlt-1 gene therapy in patients with wet age-related macular degeneration (wAMD) at 10 months post injection. 8 subjects with were randomized to treatment (6 subjects) or control (2 subjects). All subjects received 0.5 mg ranibizumab at baseline and day 30 to provide anti-VEGF therapy during the initial ramp-up period. Following this, ranibizumab was given only when a subject met criteria for re-treatment based on visual acuity (VA) and optical coherence tomography (OCT). Seven days after baseline, treated subjects received either 1E10 vg ( $n=3$ ) or 1E11 vg ( $n=3$ ) rAAV.sFlt-1, administered in 100  $\mu$ l volume via subretinal injection. Enrolled subjects had average age of  $79\pm4.6$  years, had longstanding and extensively treated wAMD, and multiple co-morbidities characteristic of the elderly population. Serial ophthalmic examinations over time revealed no superficial, anterior segment, no vitreous inflammatory signs, no significant intraocular pressure elevation, and no retinal detachment in any of the patients. Vector sequence was found in the tear samples of two patients one day after surgery that cleared by the next time point. Vector sequence was not found in other samples and AAV capsid was not detected. Subjects with neutralizing antibodies to AAV were not excluded, and 50% of enrolled subjects had neutralizing antibodies to AAV at baseline. Clinical laboratory assessments including lymphocyte subset counts did not show any significant change from baseline. 5 out of 6 patients treated with gene therapy did not require reinjection over the study period whereas 2/2 controls met re-treatment criteria and received injections over the study period. The majority of patients had improved vision. We show here that subretinal injection of rAAV.sFlt-1 is well-tolerated by elderly patients with wAMD, is safe, and potentially efficacious as a long-term treatment for wAMD following a single administration. One year follow up data on these patients will also be presented.